

Claims:

1. A method of making a plant artificial chromosome, comprising:
 - (a) preparing recombinant protoplasts of a first plant species containing an exogenous nucleic acid;
 - 5 (b) producing chromosome fragments of chromosomes contained in the recombinant protoplasts;
 - (c) fusing the recombinant protoplasts of (b) with protoplasts of a second plant species to produce fused protoplasts, wherein the first and second plant species may be the same or different; and
 - 10 (d) identifying fused protoplasts of (c) or cells derived from the fused protoplasts of (c) that contain chromosome fragments that exhibit normal plant chromosomal properties.
 2. The method of claim 1 wherein (b) comprises irradiating the protoplasts.
 3. The method of claim 1 wherein (b) comprises contacting the protoplasts with a
15 chemical agent.
 4. The method of claim 3 wherein the chemical agent is calicheamicin, esperamicin, dynemicin or neocarzinostatin.
 5. The method of claim 1 wherein said identifying of (d) comprises pulsed field gel electrophoresis.
 - 20 6. The method of claim 1 wherein said second plant species is the same as said first plant species.
 7. The method of claim 1 wherein said second plant species is a member of the same family as said first plant species.
 8. The method of claim 1 wherein said first plant species is a monocot.
 - 25 9. The method of claim 1 wherein said first plant species is a dicot.
 10. The method of claim 1 further comprising (e) regenerating a whole plant from the recombinant protoplasts of (a), prior to (b).
 11. The method of claim 1 further comprising (f) regenerating a whole plant from the fused protoplasts or plant cells identified in claim 1(d).
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12. The method of claim 1 wherein the exogenous nucleic acid comprises at least one recombination site.
13. The method of claim 1 wherein the exogenous nucleic acid comprises at least one restriction site.
- 5 14. The method of claim 1 wherein the exogenous nucleic acid comprises at least one coding region.
15. The method of claim 1 wherein the exogenous nucleic acid comprises at least one sequence encoding a yeast chromosomal element.
- 10 16. The method of claim 1 wherein the exogenous nucleic acid comprises a yeast artificial chromosome.
17. The method of claim 1 wherein the exogenous nucleic acid comprises a sequence encoding a selectable marker.
18. A method of preparing a transgenic plant comprising:
- 15 (a) preparing recombinant protoplasts of a first plant species containing an exogenous nucleic acid;
- (b) producing chromosome fragments of chromosomes contained in the recombinant protoplasts;
- (c) fusing the recombinant protoplasts of (b) with protoplasts of a second plant species to produce fused protoplasts, wherein the first and second plant species may be the same or different;
- 20 (d) identifying fused protoplasts of (c) or cells derived from the fused protoplasts of (c) that contain chromosome fragments that exhibit normal plant chromosomal properties; and
- e) regenerating a whole plant from the protoplasts or cells identified in (d) that contain said chromosome fragments exhibiting normal plant chromosomal properties.
- 25 19. A method of making a plant artificial chromosome, comprising:
- (a) producing transformed plants of a first plant species containing an exogenous nucleic acid;
- (b) producing chromosome fragments of chromosomes of said first plant species;
- 30 (c) crossing said first plant species containing the chromosome fragments with a

second plant species to produce hybrid plant species wherein said first and second plant species may be the same or different; and

(d) identifying hybrid plant species of (c) or cells or protoplasts thereof containing at least one chromosome fragment that exhibit normal plant chromosomal functions.

- 5 20. Isolated plant cells or protoplasts containing at least one plant chromosome fragment exhibiting normal chromosomal properties produced by the method of claim 1.
21. A culture of protoplasts or plant cells identified in claim 1(d).
22. A whole plant produced by the method of claim 11.
23. The whole plant of claim 22 that is a monocot plant.
- 10 24. The whole plant of claim 22 that is a dicot plant.
25. Seed derived from the whole plant of claim 22.
26. The whole plant regenerated by the method of claim 18.
27. A plant cell culture derived from the whole plant of claim 25.
28. Seed derived from the whole plant of claim 26.
- 15 29. A hybrid plant species or cells or protoplasts thereof containing at least one chromosome fragment that exhibits normal plant chromosomal functions, produced by the method of claim 19.
30. A recombinant nucleic acid comprising a first centromeric sequence functional in a plant cell and a second centromeric sequence functional in a yeast cell.
- 20 31. A recombinant vector comprising the nucleic acid of claim 30.
32. The recombinant vector of claim 30 that is a shuttle vector.
33. A recombinant cell comprising the nucleic acid of claim 30.
34. The recombinant cell of claim 33 which is a plant cell.
35. The recombinant cell of claim 33 which is a yeast cell.